(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 May 2003 (01.05.2003)

PCT

(10) International Publication Number
WO 2003/035829 A3

(51) International Patent Classification[7]: C12Q 1/68, C07H 21/00, 21/02, 21/04

(21) International Application Number: PCT/US2002/032088

(22) International Filing Date: 8 October 2002 (08.10.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/327,864 9 October 2001 (09.10.2001) US
10/008,978 7 December 2001 (07.12.2001) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 10/008,978 (CIP)
Filed on 7 December 2001 (07.12.2001)

(71) Applicant *(for all designated States except US)*: **NANOSPHERE, INC.** [US/US]; 1818 Skokie Boulevard, Northbrook, IL 60062 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PARK, So-Jung** [KR/US]; Apt. #204, 3600 North Hills Drive, Austin, TX 78731 (US). **TATON, Thomas, A.** [US/US]; 2389 Greenbrier Circle, Little Canada, MN 55117 (US). **MIRKIN, Chad, A.** [US/US]; 111 16th Street, Wilmette, IL 60091 (US). **LETSINGER, Robert, L.** [US/US]; 316 Third Street, Wilmette, IL 60091 (US). **MUCIC, Robert, C.** [US/US]; 1600 Bel Aire Dr., Glendale, CA 91201 (US). **STORHOFF, James, J.** [US/US]; 1735 Washington Street, Evanston, IL 60202 (US). **ELGHANIAN, Robert** [US/US]; 4935 West Louis Avenue, Apt. 2, Skokie, IL 60077 (US). **GARIMELLA, Viswanadham** [IN/US]; 910 Washington Street, Apt. #1C, Evanston, IL 60202 (US). **LI, Zhi** [CN/US]; 911 Shermand Avenue, Apt. 307, Evanston, IL 60202 (US). **LU, Gang** [CN/US]; 818 Noyes Street, Apt. 1A, Evanston, IL 60201 (US).

(74) Agent: **MIAO, Emily**; McDonnell Boehnen Hulbert & Berghoff, Suite 3200, 300 South Wacker Drive, Chicago, IL 60606 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 26 August 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/035829 A3

(54) Title: NANOPARTICLES HAVING OLIGONUCLEOTIDES ATTACHED THERETO AND USES THEREFOR

(57) Abstract: The invention provides methods of detecting a nucleic acid. The methods comprise contacting the nucleic acid with one or more types of particles having oligonucleotides attached thereto. In one embodiment of the method, the oligonucleotides are attached to nanoparticles and have sequences complementary to portions of the sequence of the nucleic acid. A detectable change (preferably a color change) is brought about as a result of the hybridization of the oligonucleotides on the nanoparticles to the nucleic acid. The invention also provides compositions and kits comprising particles. The invention further provides methods of synthesizing unique nanoparticle-oligonucleotide conjugates, the conjugates produced by the methods, and methods of using the conjugates. In addition, the invention provides nanomaterials and nanostructures comprising nanoparticles and methods of nanofabrication utilizing nanoparticles. Finally, the invention provides a method of separating a selected nucleic acid from other nucleic acids.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/32088

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12Q 1/68; C07H 21/00, 21/02, 21/04
US CL : 435/6; 536/23.1, 24.3, 24.33, 25.3
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6; 536/23.1, 24.3, 24.33, 25.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,214,560 B1 (YGUERABIDE et al) 10 April 2001, see entire document.	1-24, 27-42, 49-65, 95-102, 107-122, 132-134, 136-143, 487-502, 507
Y	US 6,025,202 A (NATAN) 15 February 2000, see entire document.	1-24, 27-42, 49-65, 95-102, 107-122, 132-134, 136-143, 487-502, 507

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
28 September 2003 (28.09.2003)

Date of mailing of the international search report
09 OCT 2003

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703)305-3230

Authorized officer
Jeffrey Bridges
Celia Riley

Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/32088

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-24, 27-42, 49-65, 95-102, 107-122, 132-134, 136-143, 487-502, 507

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

The inventions listed as Groups 1-33 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the inventions uses either different types of probes, labels or are directed to different methods.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1, claim(s) 1-24, 27-42, 49-65, 95-102, 107-122, 132-134, 136-143, 487-502, 507, drawn to method of detecting a nucleic acid and kit.

Group 2, claim(s) 43-48, 123, 124, 144, 145, drawn to Method of detecting a nucleic acid and kit using liposomes.

Group 3, claim(s) 49-69, 125-131, 156-161, 503-506, drawn to method of detecting using aggregate probes.

Group 4, claim(s) 70-79, 162-166, drawn to method of detection using core probes.

Group 5, claim(s) 25, 26, 80-82, 103-107, 109-1154, 132-135, drawn to method of detection using binding oligos.

Group 6, claim(s) 83-94, 108, 146-155, drawn to method of detection using energy donors.

Group 7, claim(s) 167-168, drawn to a substrate.

Group 8, claim(s) 169, drawn to semiconductor.

Group 9, claim(s) 170, drawn to a satellite probe.

Group 10, claim(s) 171-177, drawn to nanofabrication.

Group 11, claim(s) 178-184, 188, 237-265, 429, 430, 433-452, drawn to nanomaterials.

Group 12, claim(s) 185-187, drawn to assembly of containers.

Group 13, claim(s) 189, drawn to method of separation.

Group 14, claim(s) 190-236, drawn to method of binding.

Group 15, claim(s) 266-424, drawn to method of detection.

Group 16, claim(s) 425-428, drawn to nanofabrication.

Group 17, claim(s) 431-432, drawn to method of separation.

Group 18, claim(s) 453-483, drawn to method of binding.

Group 19, claim(s) 484-486, drawn to oligonucleotide.

Group 20, claim(s) 508, drawn to method of detecting a polyvalent analyte.

Group 21, claim(s) 509-519, drawn to method of detection using sbp.

Group 22, claim(s) 520-531, drawn to method of detection using *sbp* and aggregate probe.

Group 23, claim(s) 532-533, drawn to nanoparticles.

Group 24, claim(s) 534-535, drawn to aggregate probe.

Group 25, claim(s) 536, drawn to method for preparing a nanoprobe.

Group 26, claim(s) 537-564, drawn to kits.

Group 27, claim(s) 565-569, drawn to nanofabrication.

Group 28, claim(s) 570-571, drawn to method of separation.

Group 29, claim(s) 572-574, drawn to method for accelerating movement.

Group 30, claim(s) 575-598, drawn to method of detection.

Group 31, claim(s) 599-626, drawn to method of detection.

Group 32, claim(s) 627-669, drawn to method of detection using electrical labels.

Group 33, claim(s) 670-677, drawn to method for increasing stringency.

Continuation of B. FIELDS SEARCHED Item 3:
STN
search terms: nanoparticles, hybridization, arrays, probes, gold